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Bleomycin-induced chromosome breaks as a risk marker for lung cancer: a case-control study with population and hospital controls

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Environmental exposure to carcinogens and individual susceptibility play significant roles in cancer risk. Sub-optimal DNA repair capability, measured by quantifying mutagen-induced chromosome breaks, might explain variable host susceptibility to environmental carcinogens. In an ongoing lung cancer case-control study, we compared individual sensitivity to bleomycin-induced chromosome breaks in 152 non-small cell lung cancer patients with 94 population controls and 85 hospital controls with no history of cancer. Mutagen sensitivity was measured by mean number of chromatid breaks per cell in cultured peripheral blood lymphocytes treated with bleomycin. Non-parametric tests and χ^2 tests were used to determine the statistical significance of the crude case-control comparisons, followed by logistic regression to adjust for important covariates. The mean number of bleomycin-induced breaks per cell was 1.01 for the cases compared with 0.86 for hospital controls ($P < 0.01$) and 0.89 for population controls ($P < 0.01$). The mean number of breaks per cell was 1.01 for those >65 years old and 0.81 for those ≤65 years old ($P < 0.01$) among population controls. Defining bleomycin sensitive as >0.84 break/cell (the median level in population controls), 67% of the cases were bleomycin sensitive compared with 49% of the hospital controls [adjusted odds ratio (OR) = 2.69, 95% confidence interval (CI) = 1.44, 5.04], and 51% of the population controls (adjusted OR = 2.18, 95% CI = 1.13, 4.21). Our data indicate that the increased number of bleomycin-induced chromosome breaks was significantly associated with an increased risk of lung cancer in the first 331 subjects.

Introduction

Despite downward trends of lung cancer mortality in men, lung cancer mortality continues to rise in women and remains by far the leading cause of cancer-related death in men and women (1). In 2002, it is estimated that there will be 169 400 new cases of lung cancer diagnosed in the US and 154 900 people will die of this disease. Treatment advances have been modest and new strategies are needed. The 5-year survival rate has improved only from 7 to 15% in the past three decades (2). A number of risk factors for lung cancer have been identified, but a single risk factor, cigarette smoking, is responsible for >80% of the lung cancer burden (3,4). More than 90% of lung cancer patients are smokers, but the fact that only 10% of smokers develop lung cancer suggests that genetic and acquired host factors modulate susceptibility to tobacco carcinogens (5).

The ability to repair DNA lesions is strongly associated with the risk of cancer and other chronic diseases (6). Epidemiologic studies of markers of DNA repair and susceptibility to cancer in humans have revealed positive and consistent associations between DNA repair capacity and cancer occurrence (7). Inter-individual variability in human responses to carcinogens has been described repeatedly. This notion was initially supported by the rare autosomal recessive disorders such as ataxia telangiectasia, Fanconi anemia, Bloom's syndrome and Xeroderma pigmentosum, which are associated with genetic instability, defective DNA repair and increased cancer risk (8–10). Apart from these rare syndromes, individuals with variant forms of less highly penetrant genes tend to develop lung cancer at earlier ages and with lower levels of tobacco exposure than do individuals with non-susceptible genotypes (11). Epidemiologic studies of familial aggregation of lung cancer also provided indirect evidence for the role of genetic predisposition to lung cancer. Both smoking and non-smoking relatives of lung cancer patients tend to have an increased risk of lung cancer (12–15).

Cellular DNA repair capacity can be measured in several ways and several promising markers of lung cancer susceptibility have recently been identified. Of these, cytogenetics in combination with molecular biologic techniques has led to the development of the mutagen sensitivity assay (MSA). The MSA quantifies the frequency of chromatid breaks induced by bleomycin in cultured lymphocytes *in vitro* as an integrated biomarker of mutagen sensitivity and DNA repair capacity (16). The number of bleomycin-induced breaks per cell has been used to identify susceptible subjects. In a number of studies, patients with cancers of the head and neck as well as lung have been observed to express the mutagen-sensitive phenotype significantly more often than cancer-free control subjects (16–21). However, all of the case-control studies conducted so far are limited to certain subgroups of the population such as African-Americans or Mexican-Americans and have used convenient control samples recruited from community centers, cancer-screening programs, churches, employee groups and health maintenance organizations in the Houston metropolitan area, Texas. Population-based studies are needed to establish the usefulness and ability to generalize from the MSA, and to provide reliable and precise estimates of its association, if any, with lung cancer in the population.

In this study, we investigate the association of bleomycin sensitivity and lung cancer risk in a case-control study with

Abbreviation: MSA, mutagen sensitivity assay.

both hospital- and population-based control groups in Caucacians and African-Americans in the greater metropolitan area of Baltimore, Maryland. This is an on-going case-control study designed to confirm previous findings with hospital-based controls and to extend them to population-based controls. We also examined the reproducibility of the mutagen sensitivity assay and its potential use for predicting lung cancer risk.

Materials and methods

Study subjects

Lung cancer patients of Caucasian or African-American descent, residing in Metropolitan Baltimore and the Maryland Eastern Shore (all the Maryland counties east of the Chesapeake Bay) from seven hospitals in Baltimore City were recruited into the study. There were no age and stage restrictions for cases. All cases were histologically confirmed non-small cell primary tumors of the lungs. Hospital controls were cancer-free patients recruited from the same hospital as cases and were frequency matched to the cases by gender, ethnicity (Caucasian or African-American, as self-identified by the participants), age and smoking history. Population controls were recruited from Baltimore City and the same counties as the lung cancer cases by screening information obtained from the Department of Motor Vehicles (DMV) to match cases by age, gender and ethnicity. Eligible individuals from the DMV database were randomly selected for enrollment. Study subjects recruitment and matching are on-going processes and the data were reported as it is. Complete matching has not been achieved for smoking history, and age in females. Therefore, age and smoking history were adjusted in the logistic model. The study was approved by the Institutional Review Boards of the National Cancer Institute, University of Maryland, The Johns Hopkins University School of Medicine, Sinai Hospital, MedStar Research Institute and the Research Ethics Committee of Bon Secours Baltimore Health System.

Eligibility criteria

Eligible subjects had to be either Caucasian or African-American, free of known diagnosis of HIV, HCV and HBV; born in the US; a resident of Baltimore City and adjacent counties of Maryland or the Maryland Eastern Shore; able to speak English well enough to be interviewed; non-institutionalized; currently not taking antibiotics or steroid medications; never being interviewed as a control for the study (for cases only). Subjects who had undergone chemotherapy or radiation therapy were excluded from the study, and those who had undergone surgery provided a blood sample either before the surgery or 2 months after the surgery. Chemotherapy and radiation therapy are known to affect the MSA, and so we excluded such subjects to maximize the validity of the MSA results.

After informed consent was obtained, cases and controls received a structured, in-person interview assessing prior medical and cancer history, tobacco use, alcohol use, current medications, occupational history, family medical history, menstrual history and estrogen use, recent nutritional supplements and caffeine intake, and socioeconomic characteristics. Blood was obtained by the interviewers in heparinized tubes. Aliquots of the blood samples were transferred within 24 h of collection to the Laboratory of Human Carcinogenesis at National Cancer Institute for cytogenetic analyses. Laboratory personnel were masked to each participant's case-control status.

A sample of 14 subjects (seven cases and seven controls) were randomly selected from the total study population and were used in a cryopreservation test. Cryopreserving was done with 6% DMSO. Sixty microliters of DMSO were added to 1 ml of whole blood in a cryo-tube. The blood was mixed thoroughly, and then placed in a controlled freezing apparatus, that decreased temperature at 1°C/min. The blood was kept at –70°C for overnight and cultured the next day in parallel with fresh blood. For each specimen, two cultures were set-up for fresh blood and two for cryopreserved blood.

Mutagen sensitivity assays

The assay was described in detail previously (16). Briefly, 1 ml of fresh whole blood was added to 9 ml of RPMI-1640 medium supplemented with 15% bovine serum (Biofluid, Rockville, MD), 1.5% of phytohemagglutinin (Life Technologies, Rockville, MD), 2 mM L-glutamine, and 100 U/ml each of penicillin and streptomycin. After the cells were cultured for 72–90 h at 37°C, they were incubated for 5 h with 0.03 U/ml Bleomycin (Mead Johnson Oncology Products, Princeton, NJ). To arrest the cells at metaphase, 0.2 μg/ml Colcemid was added to the culture 1 h before the harvest. The cells were treated in hypotonic solution (0.06 M KCl) and fixed in fixative (three parts of methanol with one part of acetic acid). The cells were dropped onto clean microscopic slides, air dried and stained with 4% Gurr's Giemsa solution (BDH Laboratory Supplies, UK). Fifty well-spread metaphase cells per subject were examined to visually score the chromatid breaks. Only frank chromatid breaks or chromatid exchanges were scored. Criteria for a frank chromatid break were a discontinuity of a single chromatid in which the distance of discontinuity region was wider than the diameter of the chromatid or there was a clear misalignment of one of the chromatids. A chromatid exchange is the result of two or more chromatid breaks and the subsequent rearrangement of chromatid material. Exchanges may be between chromatids of different chromosomes (interchanges), or between or within chromatids of one chromosome (intrachanges). The total number of breaks was divided by the number of the cells examined and the mean number of breaks per cell was recorded for statistical analysis. Cells with more than 12 breaks were excluded from the calculation of mean breaks per cell to reduce the bias of the results by a very few severely damaged cells. In our study, the frequency of the cells with more than 12 breaks was rare. Fifty cells were analyzed from one slide without seeing one cell with more than 12 breaks for the vast majority of the subjects. The slides were coded and scored without the knowledge of case-control status.

Statistical analyses

Spearman's correlation was used to test the reproducibility of the MSA. The χ^2 goodness-of-fit test was used to examine the distributions of age, gender, race and smoking status between cases and controls. In dichotomous analyses, an individual was considered sensitive to bleomycin if the number of breaks per cell was equal to or greater than the 50th percentile of breaks per cell in population controls. To assess for the presence of a trend in lung cancer risk according to degree of mutagen sensitivity, we then analyzed the data according to ordered categories, using the quartiles of the population control as cut-off points. Multivariate logistic regression and linear regression were used to analyze the relationship between lung cancer risk and mutagen sensitive phenotype, while controlling for other covariates. The number of bleomycin-induced chromatid breaks was analyzed as a continuous variable. Age, gender and smoking status were possible confounders that were adjusted for in the multivariate analyses. Age was dichotomized based on the median age of the study subjects. Smoking status was stratified into three categories: never smoker, former smoker and current smoker. All *P* values were two-sided. All analyses were performed using SAS software, version 8 (Cary, NC).

Results

Study population

Table I summarizes selected demographic characteristics of the case and control subjects. The case and control groups were well matched on some, but not all, sampling characteristics. Among men, cases and controls were similar in mean age, but the female lung cancer patients were significantly older than female controls ($P < 0.01$). The lung cancer cases were significantly more likely than the controls to be smokers ($P < 0.01$). The gender distribution was not significantly different, but males were somewhat over-represented in the population control group. The overall participating rates of eligible individuals are 86, 85 and 19% for cases, hospital controls and population controls, respectively. There are no significant differences of distribution of gender, race, mean age and social-economic status between responders and non-responders among cases. The distribution of race, mean age and social-economic status are similar between responders and non-responders for hospital controls. However, males are significantly more likely to be the non-responders than females among hospital controls ($P = 0.005$). African-Americans are significantly more likely to be the non-responders than Caucasians among population controls ($P = 0.003$). The distributions of gender, mean age and social-economic status are very similar between responders and non-responders among population controls.

Reproducibility of MSA

To test the reproducibility of the MSA, two cultures were set up for each blood sample for the first 120 subjects. There were no differences of mean breaks per cell between culture 1 and culture 2 (0.97 ± 0.31 and 0.96 ± 0.35, respectively, $P = 0.66$). The mean breaks per cell were significantly

Table I. Distribution of selected characteristics of study subjects

	Cases, $n = 152$	Hospital controls, $n = 85$	P	Population controls, $n=94$	P
Age, mean (SD)	66.2(10.1)	61.7(12.1)	0.01	61.0(10.5)	<0.01
Male	65.6(10.7)	67.1(9.5)	0.34	63.4(10.1)	0.25
Female	66.8(9.4)	57.7(12.3)	<0.01	57.1(12.7)	<0.01
Gender, *n* (%)					
Male	77(50.7)	36(42.4)		58(61.7)	
Female	75(49.3)	49(57.6)	0.22	36(38.3)	0.09
Race, *n* (%)					
African-American	53(34.9)	25(29.4)		23(24.5)	
Caucasian	99(65.1)	60(70.6)	0.39	71(75.5)	0.09
Smoking status, *n* (%)					
Never	11(7.7)	29(34.9)		41(44.6)	
Former	64(44.8)	37(44.6)		34(37.0)	
Current	68(47.6)	17(20.5)	<0.01	17(18.5)	<0.01

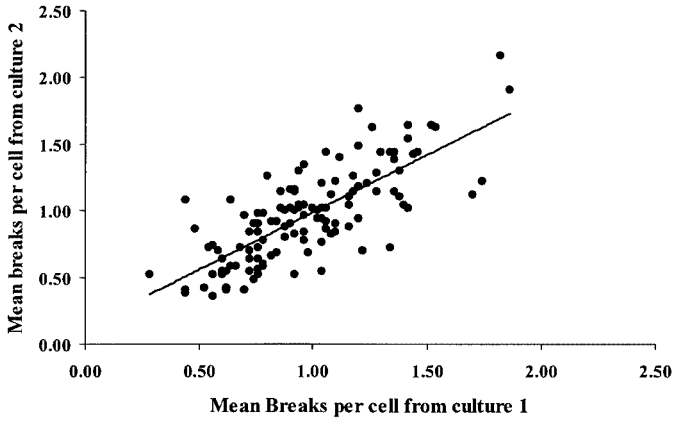

Fig. 1. Simple plot of bleomycin-induced chromatid breaks from two duplicated cultures. Fifty well-spread metaphase cells were scored to obtain the mean breaks per cell from each culture. Each dot represents one subject. The mean breaks per cell were significantly correlated between culture 1 and culture 2 (Spearman's correlation coefficient $r = 0.75$, $P < 0.01$).

correlated between culture 1 and culture 2 ($r = 0.75$, $P < 0.01$, Figure 1).

Bleomycin sensitivity and lung cancer risk

Overall, the mean number of bleomycin-induced breaks per cell was 1.01 for the cases compared with 0.86 for hospital controls ($P < 0.01$) and 0.89 for population controls ($P < 0.01$, Table II). In both sexes, the cases had higher levels of mean breaks per cell than either their hospital- or population-based counterparts, but only in women were the differences statistically significant. African-American cases and younger cases were more sensitive to bleomycin than their matched controls groups, but there were no significant case-control differences in bleomycin sensitivity among Caucasians and older subjects.

Defining bleomycin sensitive as >0.84 break/cell (median level in population controls), 67% of the cases were bleomycin sensitive compared with 49% of the hospital controls with an adjusted OR of 2.69 (95% CI = 1.44, 5.04) adjusted by age as continuous variable, gender, race and smoking status. Fifty-one percent of the population controls were bleomycin sensitive with an adjusted OR of 2.18 (95% CI = 1.13, 4.21). The risk of lung cancer was associated with an increased number of bleomycin-induced chromatid breaks in a dose-response relationship (Table III and Figure 2). The risk of lung cancer increased according to increasing quartiles of breaks per cell in both hospital (P-for-trend <0.01) and population (P-for-trend = 0.03) controls (Table III).

The bleomycin sensitivity profiles within the case and control groups were assessed by age, gender, race and smoking status (Table II). There were no statistically significant differences in bleomycin sensitivity by race and smoking status among control subjects. However, age was associated with the mean number of bleomycin-induced chromatid breaks ($r = 0.21$, $P < 0.001$). Subjects who were older than 65 years had a significantly higher number of bleomycin-induced mean breaks per cell than those younger than 65 years (1.01 and 0.81, respectively, $P = 0.002$) in population controls. A similar trend was seen in hospital controls, but not statistically significant ($P = 0.08$, Table II). However, there was no association between age and bleomycin sensitivity in cases. The frequency of mean breaks per cell in cases was significantly higher for non-smokers than for smokers (1.26 and 0.99, respectively, $P = 0.02$). We also observed that mean breaks per cell were significantly higher in males than in females among the population controls. One possible explanation is that males were significantly older than females in the population controls, but not in cases in our study (Table I), as older age was associated with an increased number of bleomycin-induced breaks.

We analyzed bleomycin sensitivity and lung cancer risk stratified by age using the population controls (Table IV). Among the younger subjects (⩽65 years old), the bleomycin sensitivity was significantly associated with lung cancer risk with an adjusted OR of 3.93 (95% CI = 1.62, 9.54). However, the bleomycin sensitivity was not associated with lung cancer risk among the older subjects (>65 years old) with an adjusted OR of 0.63 (95% CI = 0.20, 2.05). A significant dose-response relationship was observed among younger subjects (P-for-trend <0.01), but not among older subjects (P-for-trend = 0.59). We also analyzed the combined effects of bleomycin sensitivity and age. Both bleomycin sensitivity and older age are associated with an increased risk of lung cancer with an OR of 5.0 and 9.8, respectively. The combined effect of bleomycin sensitivity and age on the risk of lung cancer (OR = 7.9) was not significantly different from the single effect of bleomycin sensitivity or age (Table V).

Bleomycin sensitivity using cryopreserved lymphocytes

The rate of culture success was 100% for fresh blood and 79% for cryopreserved blood for these 14 blood samples. The

Table II. Mean bleomycin-induced breaks per cell by host characteristics

Variable	Cases		Hospital control			Population control		
	n	Mean (SD)	*n*	Mean (SD)	*P*[a]	*n*	Mean (SD)	*P*[b]
Total	152	1.01(0.3)	85	0.86(0.3)	<0.01	94	0.89(0.3)	<0.01
Gender								
Male	77	1.01(0.3)	36	0.89(0.3)	0.13	58	0.94(0.3)	0.31
Female	75	1.02(0.4)	49	0.83(0.3)	<0.01	36	0.80(0.3)	<0.01
P[c]		0.76		0.17			0.03	
Race								
AA[f]	53	1.09(0.4)	25	0.84(0.4)	0.01	23	0.83(0.3)	0.01
Caucasian	99	0.97(0.3)	60	0.86(0.3)	0.05	71	0.91(0.3)	0.19
P[d]		0.09		0.55			0.31	
Age								
⩽65	65	1.01(0.4)	43	0.81(0.3)	<0.01	58	0.81(0.3)	<0.01
>65	87	1.01(0.4)	42	0.90(0.3)	0.10	36	1.01(0.3)	0.87
P[e]		0.93		0.08			<0.01	
Smoking status								
Never	11	1.26(0.3)	29	0.89(0.3)	<0.01	41	0.91(0.3)	<0.01
Former	64	1.00(0.3)	37	0.86(0.3)	0.02	34	0.93(0.3)	0.34
Current	68	0.98(0.4)	17	0.77(0.3)	0.03	17	0.78(0.3)	0.03
P-for-trend		0.05		0.34			0.23	

[a]*P*-value for comparing the means between cases and hospital controls.
[b]*P*-value for comparing the means between cases and population controls.
[c]*P*-value for comparing the means between males and females.
[d]*P*-value for comparing the means between African-Americans and Caucasians.
[e]*P*-value for comparing the means between age ⩽65 years and age >65 years.
[f]African-American.

Table III. Risk estimates for bleomycin sensitivity

	Cases, *n*	Hospital control, *n*	Adjusted OR[a] (95% CI)	Population control, *n*	Adjusted OR (95% CI)
Dichotomized[b] (breaks/cell)					
⩽0.84	50	43	1.00	48	1.00
>0.84	102	42	2.69 (1.44, 5.04)	46	2.18 (1.13, 4.21)
By quartiles[c] (breaks/cell)					
<0.64	25	21	1.00	23	1.00
0.64–0.83	25	22	1.13 (0.44, 2.88)	23	1.37 (0.50, 3.75)
0.83–1.12	50	26	1.77 (0.75, 4.21)	26	1.96 (0.77, 4.98)
>1.12	52	16	4.19 (1.60, 10.99)	22	2.67 (1.05, 6.77)
P-for-trend			<0.01		0.03

[a]Adjusted for age as continuous variable, gender, race and smoking status (never, former, current).
[b]Dichotomized at the 50th percentile of mean breaks per cell in the population controls.
[c]Categorized by quartiles of mean breaks per cell in the population controls.

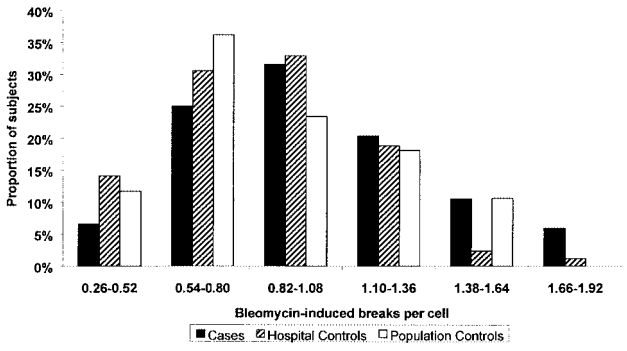

Fig. 2. Distribution of bleomycin-induced chromatid breaks by case-control status.

mean breaks per cell were 0.88 and 2.38 for fresh blood and cryopreserved blood, respectively. In cultures using fresh blood, the mean breaks per cell were 0.92 for cases and 0.82 for controls, whereas in cultures using cryopreserved blood, the mean breaks per cell were 2.31 for cases and 2.49 for controls. The mean breaks per cell were significantly higher for cryopreserved blood than for fresh blood (2.4 and 0.87 respectively, $P < 0.001$).

Discussion

The failure to maintain genome integrity is central to the problem of carcinogenesis. Increased genetic instability, either spontaneous or mutagen-induced, has been considered a predisposing factor for neoplastic transformation. Several case-control studies have indicated that bleomycin sensitivity is an independent risk factor for tobacco-related cancers (22–27).

Table IV. Risk estimates for bleomycin sensitivity between cases and population controls, stratified by age

	Age ⩽65 years old			Age >65 years old		
	Cases, *n*	Controls, *n*	OR[a] (95% CI)	Cases, *n*	Controls, *n*	OR (95% CI)
Dichotomized[b] (breaks/cell)						
⩽0.84	23	36	1.00	27	10	1.00
>0.84	42	22	3.93 (1.6, 9.5)	60	26	0.63 (0.2, 2.1)
By quartiles[c] (breaks/cell)						
<0.64	10	18	1.00	15	5	1.00
0.64–0.83	13	18	2.27 (0.7, 7.92)	12	5	0.62 (0.1, 4.7)
0.83–1.12	20	10	7.18 (1.8, 28.0)	30	16	0.42 (0.1, 2.1)
>1.12	22	12	5.50 (1.6, 18.9)	30	10	0.63 (0.1, 3.3)
P-for-trend			<0.01			0.59

[a]Adjusted by gender, race and smoking status.
[b]Dichotomized at the 50th percentile of mean breaks per cell in the population controls.
[c]Categorized by quartiles of mean breaks per cell in the population controls.

Table V. Combined effect of bleomycin sensitivity and age on lung cancer risk between cases and population controls

Bleomycin sensitive	Age	OR (95% CI)
No	No	1.0
Yes	No	5.0 (2.0, 12.5)
No	Yes	9.8 (3.1, 30.8)
Yes	Yes	7.9 (3.2, 19.4)

Age: no, 65 years old or younger; yes, older than 65 years.

In a case-control study of lung cancer in African-Americans and Mexican-Americans, the overall odds ratio for bleomycin sensitivity, after adjusting for ethnicity and smoking status was 4.3 (28). To our best knowledge, our study is the first independent study to confirm that increased sensitivity to bleomycin is associated with an increased risk of lung cancer in both Caucasians and African-Americans. We also extended the findings in this field by including population controls, and our data further support the findings that bleomycin sensitivity is associated with lung cancer risk, while minimizing the possible selection bias associated with the use of convenience controls recruited from community centers, cancer-screening programs, churches, employee groups and health maintenance organizations. However, like any other epidemiologic studies, there are limitations in our study. The low participating rate in population control may have introduced some potential bias. In our study, the distribution of gender, social-economic status and mean age are very similar between responders and non-responders among population controls. This fact provided some insurance of the quality of the population controls in our study.

The reproducibility of the bleomycin sensitivity assay has not been reported in large numbers of subjects. There are data indicating that scoring of 50 metaphases, rather than more is sufficient and that the correlation coefficient for mean break per cell values of the first and second sets of 50 readings is 0.72 (29). Some other possible sources of variability in the MSA have not been examined. For example, reader variability, culture variability and sampling time could affect MSA results. McIntyre *et al.* (30) studied reader variability of the bleomycin sensitivity assay and found that the k value (Kappa statistic) of score agreement was fair (0.4–0.6) with the same reader and was poor (<0.4) between readers (30). They also found that inter-reader agreement dramatically increased if meetings between readers were held frequently and on a regular schedule. We investigated the reproducibility of the assay by duplicating the cultures for the first 120 subjects. Our results indicated that the bleomycin sensitivity assay was reproducible in our laboratory. The agreement of mean score chromatid breaks between culture 1 and culture 2 was 75%. However, we were not able to distinguish the source of variability, i.e. slide-reading or culture condition variation. We are also in the process of further validation of the bleomycin sensitivity assay by comparing repeated samples for the same individual over time, and by assessing the inter- and intra-reader variations between laboratories.

Cryopreservation is useful for preserving valuable epidemiological specimens and provides important resources for many studies. It is of interest to investigators to know whether cryopreserved lymphocytes can be used in bleomycin sensitivity assays. We found that bleomycin-induced chromosomal breaks are significantly more common in cryopreserved lymphocytes than in fresh lymphocytes. It seems that the cryopreservation procedure itself affects the sensitivity of lymphocytes. Our results also suggested that the increase in chromatid breaks is disproportional for controls (3-fold increase of mean breaks per cell) compared with cases (2.5-fold increase of mean breaks per cell). Similar results were obtained by Dr Wu's group at M.D. Anderson Cancer Center, Texas (personal communication). Our preliminary results suggested that it is necessary to establish a side-by-side comparison between the use of fresh and cryopreserved lymphocytes for a specific assay before applying the method to larger epidemiological studies.

The incidence of cancer increases with age, peaks around 85 years of age and persists very high at least up to 95 years (31–33). Currently, in the USA, ~50% of all neoplasms affect the 12% of the population older than 65 years. Older persons are more susceptible to cancer, because age-related molecular changes represent intermediate carcinogenic stages (32,34). Immune function and DNA repair efficiency both decrease with age, which reduces protection against environmental carcinogens. Our finding that increased bleomycin sensitivity is associated with an increase in age is consistent with the notion of increased susceptibility to environmental carcinogens in older people. Our data also suggested the lack of association of bleomycin sensitivity with lung cancer risk in older people (age >65 years). We do not really have a good model to

explain this observation. One possible hypothesis is that acquired host susceptibility to lung cancer may be the dominant risk factor for lung cancer in older people, and is related to environmental exposure. The cases and controls were recruited from the same geographic area and matched or adjusted on smoking (the dominant environmental exposure for lung cancer) and other variables. Thus the cases and controls may experience similar environmental exposure in our study set, which lead to the failure to detect the differences of acquired host susceptibility in lung cancer between cases and controls in older people. Future studies are needed to confirm this preliminary observation.

In summary, biomarkers that measure different endpoints can provide insight into the underlying mechanisms of carcinogenesis and provide critical information of risk assessment. In our preliminary results, bleomycin sensitivity was consistently associated with a significantly increased risk of lung cancer in both hospital and population controls and exhibited a consistent monotonic dose-response relationship with lung cancer risk. Bleomycin sensitivity thus appears to contribute to lung cancer susceptibility.

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